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Research paper

Synthesis, characterization and antimicrobial activity of Mn(ii) and Zn(ii) complexes with leucine and alanine

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Abstract

Background: Transition metals are essential metallic element and they exhibit great biological activity. In recent time, researches have shown significant progress in the utilization of transition metal complexes as drugs for treatment of several human disorders like cancer, infections and neurological disorders, amongst others.
Objective: The study attempted to obtain neutral complexes of $MLn.nH_2O$ type (M = Mn/Zn and L = leucine/alanine), characterize, and then study their biological activities.
Methods: 2mmols of the ligands were dissolved each, in 20 ml distilled water and 0.33 ml of 30% NaOH was added. Then 1 mmol of the metal salts was dissolved in 2 ml of water and stirred, the salt solution was added to the solution of the ligand and stirred. The dark brown and white precipitates for manganese amino acid complex and zinc amino acid complexe respectively were filtered off, washed, dried in air and then weighed to establish the percent of complexation. The complexes were then subjected to both spectroscopic and non-spectroscopic method of analysis.
Result: The result suggested the coordination of the ligand to the central metal.
Conclusion: The results suggested monoclinic crystal system for all the complexes formed. The antimicrobial studies showed an enhanced biological sensitivity for the complexes when compared with that of the ligands, and the structure of the newly synthesized complexes were proposed to be octahedral for Mn-amino acid and tetrahedral for the Zn-amino acid.

Keywords: Antimicrobial Activities; Characterization; Molar Conductivity; Synthesis; X-Ray Diffraction.

1. Introduction

Transition-metal complexes are interesting and exciting group of compounds having a wide range of reactivity, physical, and chemical properties (Blyth *et al.*, 2005). The study of the coordinated systems of metal-amino acids has become increasingly important in recent times and from different points of view, that is, as some researchers are interested in the catalytic properties these complexes, some the antimicrobial properties, some the antitumoural properties, antioxidant properties among others (Al-Noor *et al.*, 2013). Recent researches have shown significant progress in the utilization of transition metal complexes as drugs for treatment of several human disorders like cancer, lymphomas, infections, inflammation, diabetes, neurological disorders, etc. Transition metal complexes have also been used as anti-inflammatory and anti-arthritic agents (Yiase *et al.*, 2014). These metals are essential metallic element, they exhibit great biological activity, and they participate in oxygen transport, electronic transfer reactions or the storage of ions when associated with certain metal-protein complexes (Choudharya *et al.*, 2011).
The most common biologically active metals are the late first row transition metals (Mn to Zn) (Papish *et al*., 2006). Coordination complexes of transition metals have been widely studied for their antibacterial, antifungal and potential cytotoxic chemotherapeutic agents. They have been evaluated against several pathogenic fungi and bacteria with promising results (Johari *et al*., 2009).

2. Materials and methods

1.2. Materials

Fourier-transform Infrared Spectrophotometer (FT-IR), X-ray Powder Diffractometers, pH meter, thermometer, furnace, petri dish, oven, melting point apparatus, magnetic stirrer, gouy, balance, water bath, crucible, water condenser, and digital weighing balance. All reagents and chemicals used are of analytical grade, and they include the following; Metal salts ($ZnSO_4.7H_2O$ and $MnSO_4.4H_2O$), Amino acids (Leucine and Alanine), Sodium hydroxide, Dimethylsulfoxide (DMSO) and Ethanol.

2.2. Methods

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The purpose of this experiment was to obtain neutral complexes of $ML_2.nH_2O$ (M = Mn and Zn) type, in the presence of a strong basis (NaOH) to obtain the ionization conditions of the amino acid.
The complexes were prepared in the inorganic laboratory of chemistry department Ahmadu Bello University Zaria, and complexes were prepared by following the standard procedure adopted by (Braicu, *et al.*, 2011). 2mmols of all the ligands were dissolved each, in 20 ml distilled water and 0.33 ml of 30% NaOH was added for deprotonation of the amino acids. Then 1 mmol of the metal salts was dissolved in 2 ml of water and stirred for complete dissolution of the salt, the solution of the salt was then added to the solution of the deprotonated amino acid under continuous stirring for several minutes, the resulting solution was transferred unto the magnetic stirrer for further stirring (about an hour). The dark brown and white precipitates for manganese amino acid complex and zinc amino acid complexes respectively were filtered off, washed several time with distilled water, dried in air and weighted to establish the percent of complexation.

3. Result and discussion

Table 3.1: Physical Parameter of the M(II) Complexes

Complexes	Molecular weight($gmol^{-1}$)	Melting point (^0C)	Colour	Yield ($^0/_0$)
$[Mn(L_1)_2(H_2O)_2]$	269.13	282-285	Dark brown	34
$Mn(L_2)_2(H_2O)_2$	354.34	300-302	Dark brown	47
$Zn(L_1)_2H_2O$	261.56	113-114	White	55
$Zn(L_2)_2H_2O$	346.77	307-308	white	76

L_1 = alanine ($C_3H_7NO_2$) and L_2 = leucine ($C_6H_{13}NO_2$).

Table 3.2: Solubility of the Complexes

Complex	Water	Ethanol	Ethyl acetate	DMSO
Mn- L_1	IS	SP	SS	SS
Mn- L_2	IS	SP	SS	SS
Zn- L_1	IS	IS	IS	SS
Zn- L_2	IS	IS	IS	SS

IS = Insoluble, SP = Sparingly soluble, SS = Soluble, DMSO = Dimethyl sulphoxide.

Physical parameters of the M(II) complexes.
Table 3.1 shows the physical parameters such as molecular weight, melting point (m.pt), and colour of the complexes. From this table, the information regarding the electronic structure of the complexes could be derived. The dark brown colour of Mn(II) complexes suggest presence of unpaired electron in the metal electronic orbital, while the white colour for the two Zn(II) complexes is an evidence for a filled d-orbital (a d^{10} configuration) for the metal electronic structure. Table 3.2 shows the degree of solubility of complexes in various solvent. The solubility test was carried out in order to find out the best solvent for the dissolution of the complexes, and they were found to dissolve best in DMSO.

Table 3.3: Magnetic Susceptibility and Molar Conductivity measurement

Complexes	μ_{eff}(B.M)	Temperature (K)	$\Lambda(\Omega^{-1}\ mol^{-1}\ cm^{-2})$
Mn- L_1	5.67	299	17.7
Mn- L_2	5.21	299	17.3
Zn- L_1	0.00	299	15.7
Zn- L_2	0.00	299	15.1

Magnetic susceptibility measurement and molar conductivity
Magnetic moment value 5.67 and 5.21 BM match with standard value (5.92 BM) corresponding to octahedral geometry for Mn(II) complexes and agree with the findings of (Annapure *et al.*, 2016). The 0.00 BM for the Zn(II) complexes has confirm the d^{10} configuration of the.
The metal (II) complexes were dissolved in DMSO and the molar conductivity of 10^{-4} M of their solution at room temperature was measured. The low conductance values of the complexes support the non-electrolytic nature of the complexes (Chondhekar *et al.*, 2015).

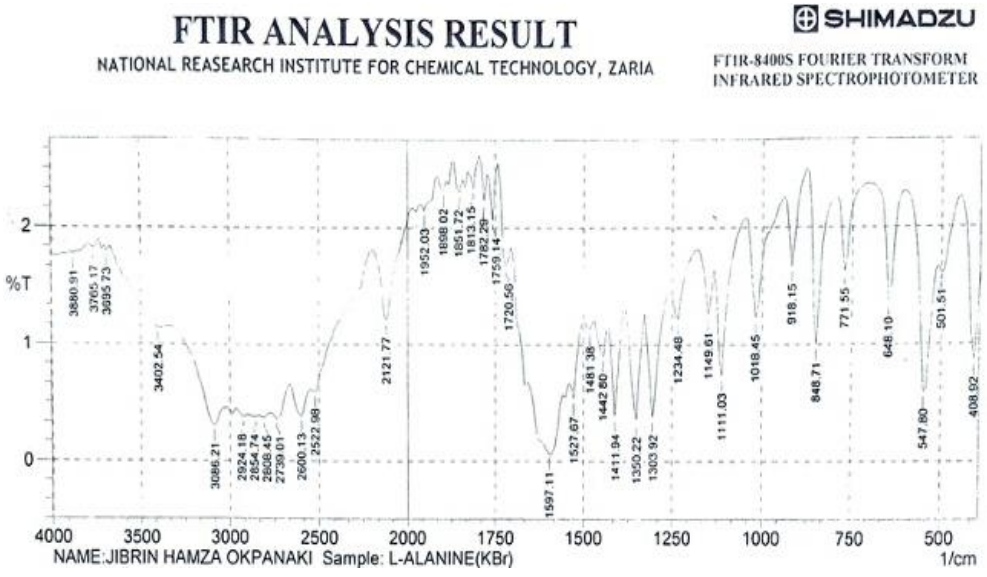


Fig. 3.1: FT- IR Spectra of L_1.

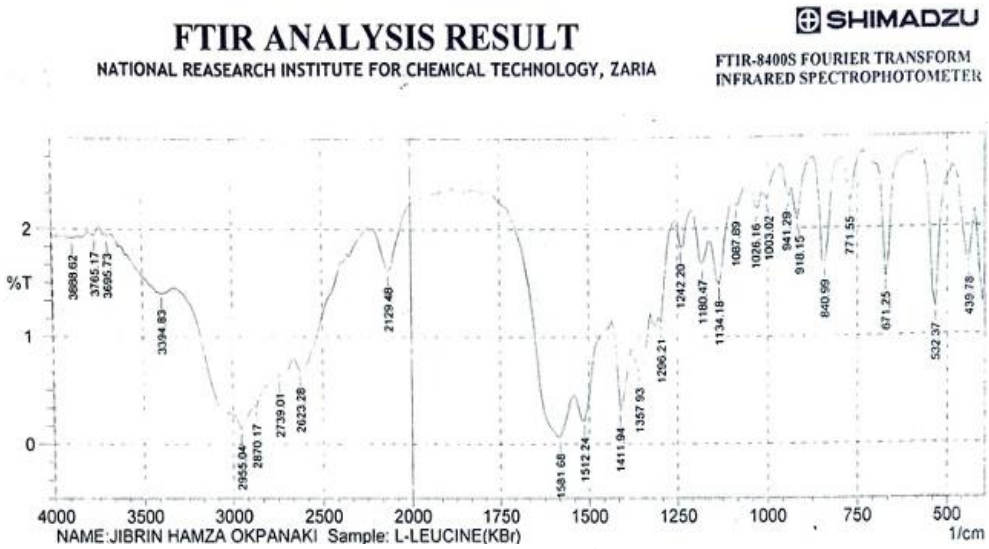


Fig. 3.2: FT- IR Spectra of L_2.

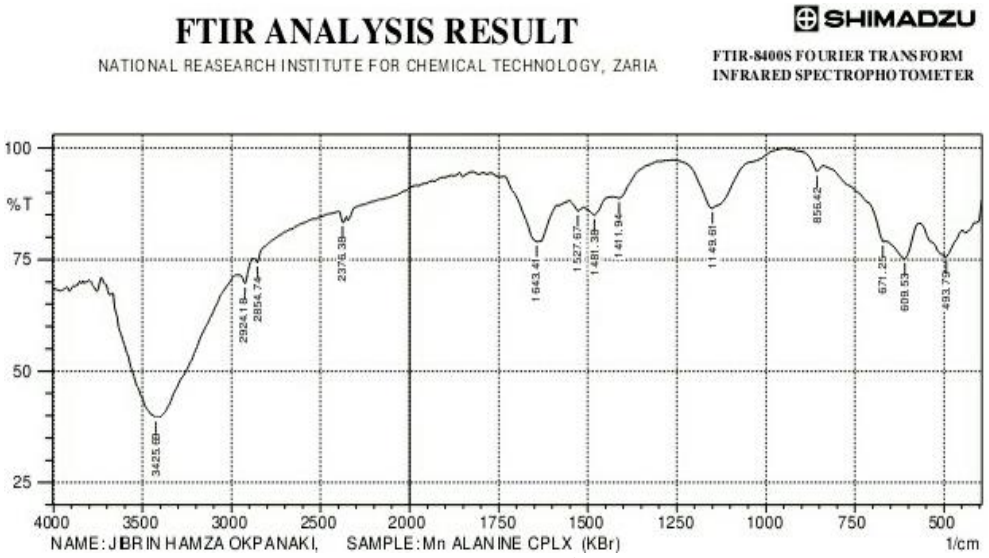


Fig. 3.3: FT-IR Spectra of Mn-L_1.

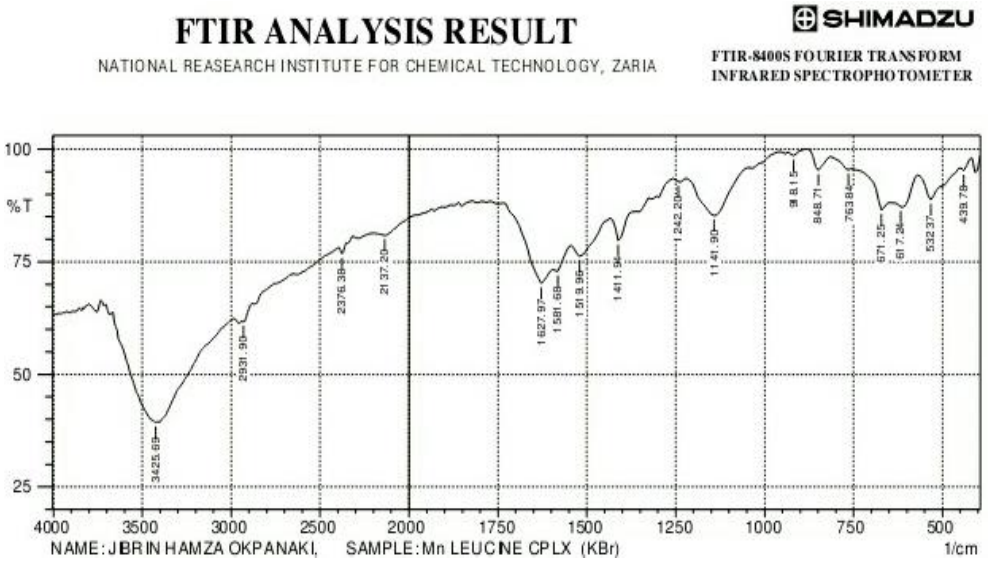


Fig. 3.4: FT-IR Spectra of Mn-L_2.

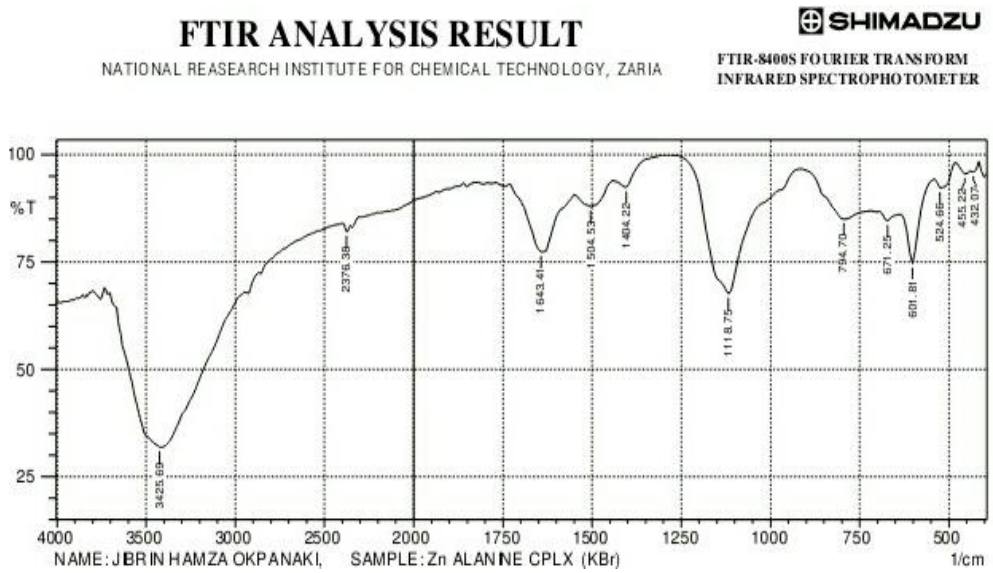


Fig. 3.5: FT-IR Spectra of Zn-L_1.

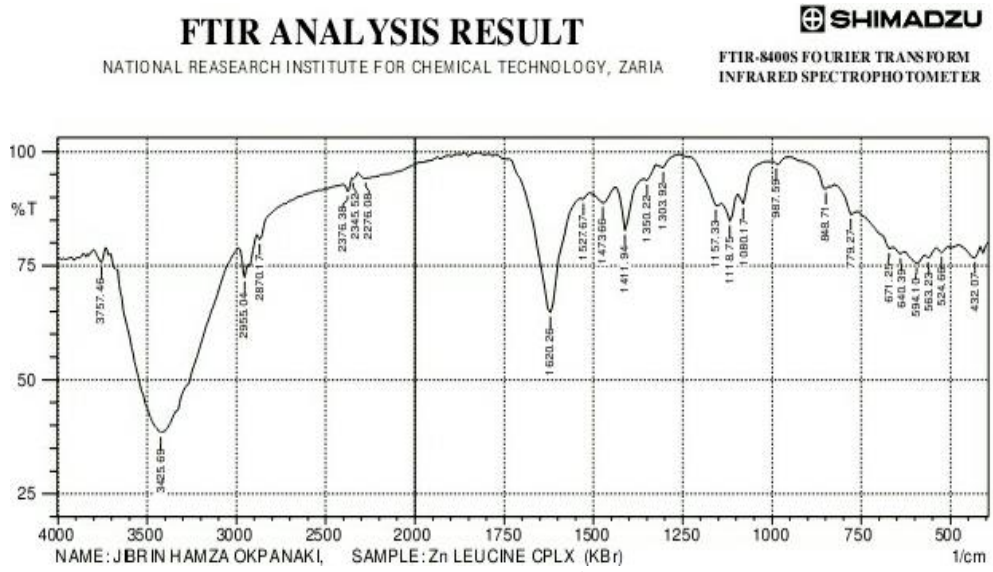


Fig. 3.6: FT-IR Spectra of Zn-L_1.

Table 3.4: Important IR Frequencies (Cm^{-1}) of $L_1$and $L_2$and Their Metal Complexes

S/N	Ligands/Complexes (cm^{-1})	υ (OH)[a]	υ(OH)[b]	υ(N-H)	asymυ(COO^-)	symυ(COO^-)	υ(M-O)	υ(M-N)
1	L_1	3402		3086	1597.	1527		
2	L_2	3394		2955	1581	1512		
3	Mn- L_1		3425	2924	1643	1481	609	493
4	Mn- L_2		3425	2931	1627	1411	617	532
5	Zn- L_1		3425	2956	1643	1504	794	601
6	Zn- L_2		3425	2953	1620	1411	779	640

[a]**OH** from amino group, [b]**OH** from water.

Fourier-transform infrared spectra

Only the most important infrared spectral bands of the ligands and complexes that provide structural evidence for the coordination of the ligands to the central metal ions are considered.

These important bands have been summarized in table 3.4 above. The FTIR spectra of the ligands were compared with these of the metal complexes in order to study the binding mode of the amino acids to the metal ions (Al-noor *et al*., 2013). Prominent bands at the region of 3403 cm^{-1} and 3394 cm^{-1} in the two ligands L_1 and and L_2 respectively are assigned to O-H stretching vibration, and this is in good agreement with the findings of (Dharwdkar *et al.,* 2003). There are broad bands that emerged at 3425 cm^{-1}in all the complexes suggest the presences of crystal water in the complexes, and this agree with was represented by (Cozma *et al*., 2009). The two bands in the ligands spectra at around 3086 cm^{-1} and 2955 cm^{-1} were found to have shifted to 2924- 2956 for ML_1 complexes and 2931-2953 cm^{-1} suggest the coordination of amino group through the nitrogen atom of the amino acid to the central metal ion. The asym (COO^-) vibration of the free amino acids i.e 1597 and 1581 cm^{-1} where shifted to a higher wave number i.e the range of 1620 cm^{-1} – 1643 cm^{-1} and the sym (COO^-) mode observed at 1512 cm^{-1} -1527 cm^{-1} in the spectra of the amino acid is found to have shifted to a lower wave number, that is 1411 cm^{-1} – 1481 cm^{-1} in the spectra of the complexes indicating the coordination of carboxylic acid group via oxygen with the metal (Dharwdkar *et al*., 2003). This may be explained on the bases of the drift in the lone pare of electrons in the two groups of the amino acids towards the metal (Kumar *et al.,* 2010). Finally some new bands of weak intensity observed in the region of 609 cm^{-1} -779 cm^{-1} and 493 cm^{-1} -640 cm^{-1} may be ascribed to the M-O and M-N vibration respectively (Dharwdkar *et al*., 2003).

Table 3.5: Analysis of Water Content

Complexes	m of ML.nH$_2$O)(g)	m of (ML) (g)	m of (H$_2$O) (g)	M$_L$ of (H$_2$0)
Mn(L$_1$)$_2$.nH$_2$O	0.00123	0.001	0.000232	3
Mn(L$_2$)$_2$.nH$_2$O	0.00117	0.001	0.000170	3
Zn-(L$_1$)$_2$.nH$_2$O	0.01140	0.010	0.001400	2
Zn-(L$_2$)$_2$.nH$_2$O	0.01109	0.010	0.001090	2

m = mass, M$_L$ = molar mass, L$_1$ = alanine and L$_2$ = leucine.

Analysis of moisture contents

The moisture content analysis was carried out to determine the amount of water present in the complexes as suggested by the FTIR analysis, and the result revealed that two and three moles of water contained in Zn (II) and Mn(II) complexes respectively.

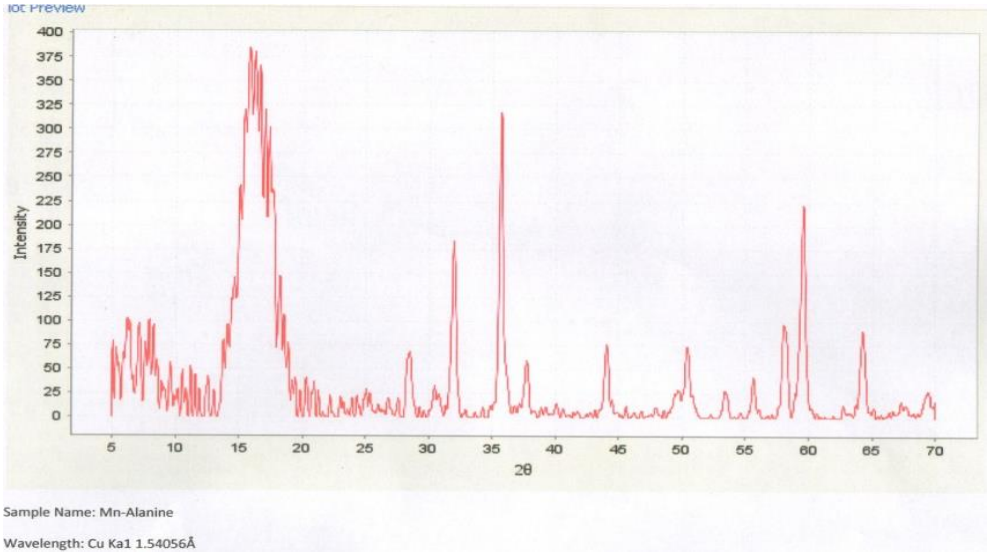


Fig 3.7: X-Ray Diffraction Spectrum of Mn- L$_1$.

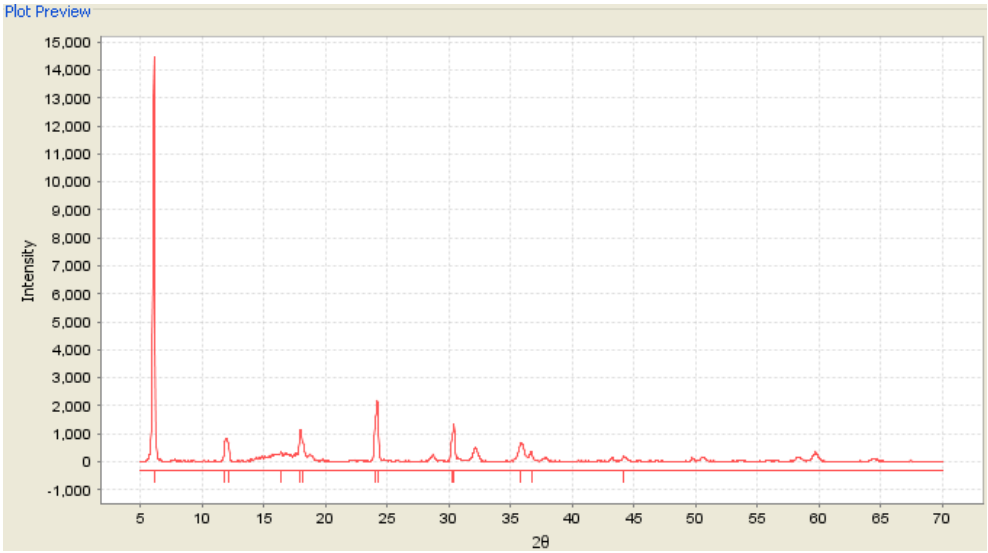


Fig. 3.8: X-Ray Diffraction Spectrum of Mn- L$_2$.

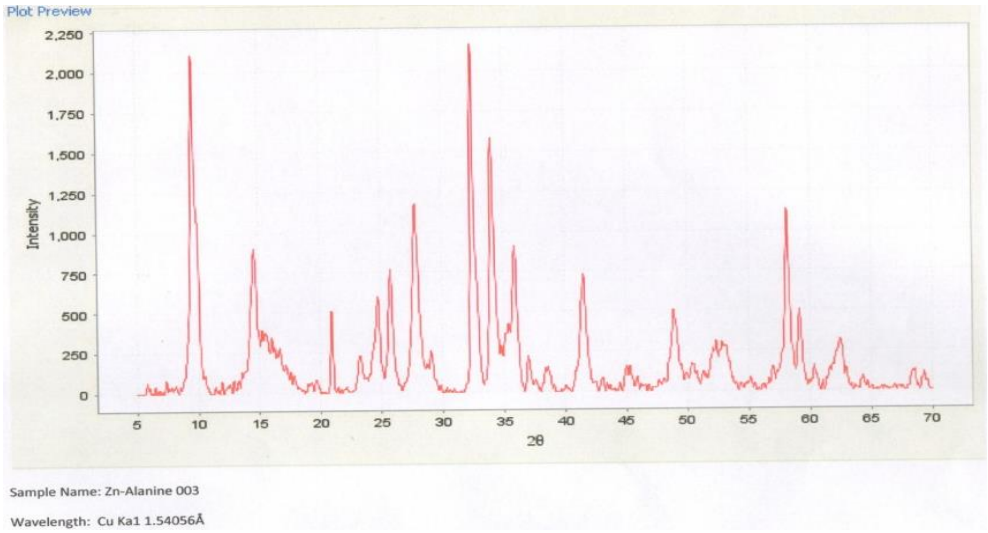


Fig. 3.9: X-Ray Diffraction Spectrum of Zn- L_1.

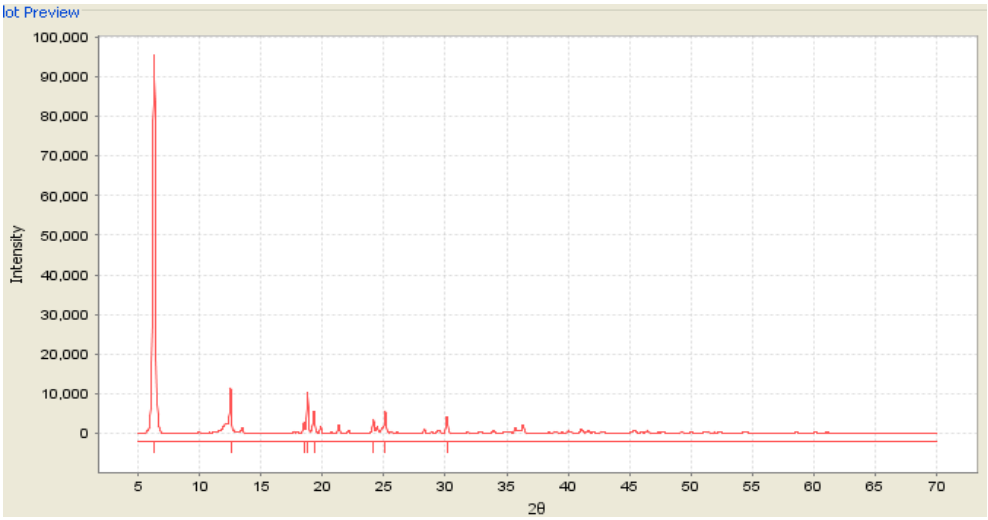


Fig 3.0: X-Ray Diffraction Spectrum of Zn- L_2.

Table 3.6: Powder X-Ray Diffraction Analysis (Lattice Parameters)

Parameters	a(Å)	b(Å)	c(Å)	α(⁰)	β(0)	γ(0)	τ(nm	V(Å)	System
Complexes									
Mn- L_1	13.49	6.010	11.41	90.0	105	90	98.0	925.06	monoclinic
Mn- L_2	14.00	5.580	12.01	90.0	98.0	90	67.0	938.22	monoclinic
Zn- L_1	10.98	15.21	8.920	90.0	109	90	121	1489.69	monoclinic
Zn- L_2	5.960	24.26	8.980	90.0	110	90	116	1298.41	monoclinic

Powder X-ray diffraction (XRD) studies

In order to test the degree of crystallinity of the synthesized complexes, The x-ray diffractogram of the M(II) complexes were scanned at the wavelength of 1.5056 Å and machine constant of 0.9.The powder XRD pattern of Mn(II)-L_1 complex showed twelve reflections in the range 16- 65° (2θ), arising from the diffractions of X-ray by the planes of complex. That of Mn(II)-L_1 complexes showed twenty peaks in the range of 5-60^0 (2θ). Similar Zn(II) complexes were scanned at the same wavelength and machine constant, there was thirty one reflections for Mn(II)-L_1 complex in the range 9-70° (2θ) and fifty seven reflections in the range 9-70^0 (2θ) for Zn(II)-leucine complex. The x-ray diffraction pattern of these complexes with respect to major peaks of relative intensity greater than 10% has been indexed by using computer program.

The average crystal sizes were found to be 98 nm, 67 nm, 121 nm and 116 nm for Mn-L_1, Mn-L_2, Zn-L_1 and Zn- L_2 respectively. The unit cell parameters for the complexes were found to be

a = 13.49 Å, b = 6.01 Å, and c = 11.41 Å and unit cell volume V=925.06 Å. α = 90^0, β = 105^0 and γ=90^0 for Mn-L_1 complex. a = 14.0 Å, b = 5.58 Å, and c = 12.01 Å and unit cell volume V=938.22 Å.α = 90^0, β = 98^{0}and γ=90^0 for Mn-L_2 complex. (Annapure et al., 2016). a = 10.98 Å, b = 15.21 Å, and c = 8.92 Å and unit cell volume V=1489.69 Å.α = 90^0, β = 109^0 and γ=90^0 for Zn-L_1 complex. a = 5.96 Å, b = 24.26 Å, and c = 8.98 Å and unit cell volume V=1298.41 Å.α = 90^0,β = 110^0 and γ =90^0 for Zn-L_2 complex. In respect of these cell parameters, the condition such as a ≠ b ≠ c and α = γ = 90° ≠ β required for sample to be monoclinic were tested and found to be satisfactory. Hence it can be concluded that the synthesize M(II) complexes of L_1 and L_2 all have monoclinic crystal system. The disagreement as regards the crystal structure between this work and the findings of (Chondhekar, et al., 2015) must have resulted from the different components of the of the complexes (metal centre and the ligand) in the two works. There is a reasonable agreement between this work and the work of (Annapure et al., 2016), though differ slightly when it comes to the individual unit cell parameters.

Table 3.7: The Antimicrobial Activity of Ligansand the Control

Organisms	L_1	L_2	Cypro	Fluconazole
MRSA	S	S	S	R

S. aureus	R	R	S	R
VRE	R	S	R	R
E. coli	S	R	S	R
P. mirabilis	S	S	S	R
S. typhi	S	S	S	R
P. arugenosa	R	R	R	R
C. albican	S	S	R	S
C. krusei	R	S	R	S
C. stellatoidea	S	R	R	S

KEY➔ S = Sensitivity, R = Resistance, Cypro = Cyprofloxacine.

Table 3.8: Zone of Inhibition of the Ligands and the Control againstthe Test Microbes

Organisms	L_1	L_2	Cyprofloxacine	Fluconazole
MRSA	20	18	31	0.0
S. aureus	0.0	0.0	34	0.0
VRE	0.0	21	0.0	0.0
E. coli	18	0.0	38	0.0
P. mirabilis	17	18	30	0.0
S. typhi	19	19	40	0.0
P. arugenosa	0.0	0.0	0.0	0.0
C. albican	20	19	0.0	0.0
C. krusei	0.0	18	0.0	35
C. stellatoidea	21	0.0	0.0	31

Table 3.9: The antimicrobial Activity of Mn-L_1, Mn-L_2, Zn-L_1, and Zn-L_2

Organisms	Mn-L_1	Mn-L_2	Zn- L_1	Zn- L_2
MRSA	S	S	S	S
S. aureus	S	S	R	S
VRE	R	R	R	R
E. coli	S	S	S	S
P. mirabilis	R	R	S	R
S. typhi	S	R	S	S
P. arugenosa	S	S	R	S
C. albican	S	S	S	S
C. krusei	S	R	R	S
C. stellatoidea	R	S	S	R

KEY➔ S = Sensitivity, R = Resistance.

Table 4.0: Zone of Inhibition of the Complex

Organisms	Mn-L_1	Mn-L_2	Zn-L_1	Zn-L_2
MRSA	24	27	24	24
S. aureus	23	21	0.0	23
VRE	0.0	0.0	0.0	0.0
E. coli	28	24	26	28
P. mirabilis	0.0	0.0	25	0.0
S. typhi	25	0.0	28	25
P. arugenosa	27	20	0.0	27
C. albican	24	26	23	24
C. krusei	20	0.0	0.0	26
C. stellatoidea	0.0	23	20	0.0

Antimicrobial activity of the ligands and the complexes

The biological activity or the therapeutic ability of a substance depends on the minimum amount by which the substance is required to inhibit the growth or kill the micro organism on which it is tested against. The in vitro antimicroial activity test of all the ligands and the newly synthesized complexes were therefore studied in serial diluted solution (200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5µg/ml) of the samples in DMSO. The activity of the ligand and the control was determined, the ligands unlike the control were found to show very low activity on the microbes, as many of them (the microbes) were found to be resistant to test samples. The ligands were found to have shown as low as 20 mm and 21 mm zone of inhibition for L_1 and L_2 respectively. The two ligands were mostly found to have MIC at 100 µg/ml 4.9, and of course very low MBC/MFC which are mostly found around 200 µg/ml for the both ligands.

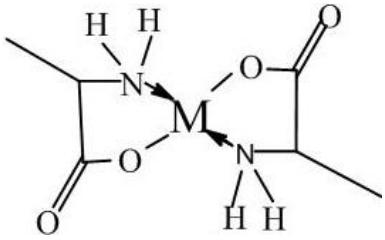
Table 3.9 shows the antmicrobial activity for M(II) complex. They were found to shown relatively better activity as many of the organisms are susceptible to the complexes with only few of them showing resistance. It could be seen in table 4.0 that the complexes have relatively higher activity as compared to the ligands, as both of the complexes exhibited zone of inhibition at 28 mm and above, with MIC spreading between 25 and 50 for the complex. The MBC was found to be around 50 and 100 , and is in agreement with the work of (Ajil *et al*, 2016) and (Annapure *et al* 2016).

Generally speaking, it is clear that the inhibition by metal complexe is higher than that of the ligand and results are in good agreement with previous findings with respect to comparative activity of free ligands and the complexes. Such enhanced activity of metal complexes is due to the increased lipophilic nature of the metal ions in complexes. The increase in activity with concentration is due to the effect of metal ions on the normal cell (An- noor *et al*., 2013).

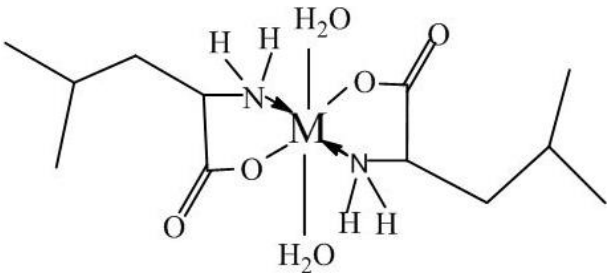
4. Conclusion

The colour and the magnetic susceptibility of the complexes have given us insight on distribution pattern of electronic structure of the complexes. The FT-IR spectra of the complexes when compared with that of the ligands suggested a bond formation between M and the carboxylic oxygen and between M and nitrogen of the amino group. The water contents analysis of the complexes also suggested similar geometry as suggested by other techniques.

The information regarding the crystal sizes and volumes has been derive from the powder XRD analysis spectra, it also suggested monoclinic crystal system for all the complexes formed. The antimicrobial activity studies showed an enhanced biological sensitivity for the complexes when compared with that of the ligands.
This is the proposed structure of the newly synthesized complexes



M(II)-L1



M(II)-L2

M(II) = Mn and Zn
L1 = alanine (C3H7NO2)
L2 = leucine ($C_6H_{13}NO_2$).

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